Evidence for histamine-mediated sensitization of TRPV1 signaling in sensory neurons in mice and IBS patients

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Background & Aims: Mast cell activation and neuronal sensitization through TRPV1 have been proposed to underlie visceral hypersensitivity in patients with irritable bowel syndrome (IBS). We recently showed that 12 weeks of treatment with the H_1R antagonist ebastine significantly improved abdominal pain and global relief compared to placebo. As histamine released by mast cells activates afferent nerves through histamine 1 receptors (H_1R), and we recently showed increased pain responses to rectal application of capsaicin in IBS, we hypothesized that histamine could be one of the mediators sensitizing TRPV1, a mechanism prevented by H_1R blockade and potentially contributing to the therapeutic effect of ebastine.

Methods: The submucosal plexus was isolated from rectal biopsies taken from 16 healthy volunteers (HV) and 12 IBS patients and responses to exogenously applied capsaicin (0.1, 1 and 10 nM) and histamine (1, 10 and 100 µM) were monitored using calcium (Ca^{2+})-imaging. In addition, the effect of 10 µM histamine pre-incubation on the capsaicin response was studied in HV. In parallel, murine dorsal root ganglia (DRG) were isolated to study the calcium response to capsaicin (10 – 250 nM) in the presence of histamine or after overnight incubation with mucosal biopsy supernatant from HV and IBS patients. Pyrilamine (1 µM) was used as H_1R antagonist.

Results: Application of histamine and capsaicin evoked significantly higher peak amplitudes in submucosal neurons from IBS patients compared to HV (Table 1). Pretreatment with

histamine significantly increased the peak amplitudes in response to capsaicin in submucosal neurons from HV (Table 1). This sensitization of TRPV1 by histamine was confirmed in mouse DRG neurons, an effect that was prevented by preincubation with pyrilamine (Table 2). In parallel studies, overnight incubation with HV supernatant spiked with 10 µM histamine significantly increased the response to capsaicin compared to control HV supernatant. Similarly, overnight exposure of DRG neurons to IBS supernatant significantly increased the capsaicin response, an effect that was reduced by pyrilamine (Table 2).

Conclusion: We showed that submucosal neurons of IBS patients are more intensely activated by capsaicin than those of HV. This effect can be mimicked by preincubation with histamine in both human submucosal neurons and mouse DRG neurons. Furthermore, incubation of mouse DRG neurons with IBS supernatant also increases the response to TRPV1, an effect that is blocked by the H_1R antagonist pyrilamine. Based on these data, we conclude that histamine sensitizes TRPV1 on sensory neurons, most likely contributing to increased visceral pain perception in IBS. Moreover, we hypothesize that the therapeutic effect of the H_1R antagonist ebastine in IBS patients can at least partly be explained by interference with this pathway.

Table 1 Submucosal neuronal activation in rectal biopsies (% response)			
Histamine	HV	IBS	Unpaired T-test
1 µM	0.66 ± 0.19 (n = 8)	0.54 ± 0.10 (n = 9)	p = 0.77
10 µM	0.60 ± 0.10 (n = 14)	1.44 ± 0.45 (n = 12)	p = 0.03
100 µM	1.02 ± 0.26 (n = 15)	1.70 ± 0.48 (n = 12)	p = 0.11
Capsaicin	HV	IBS	Unpaired T-test
0.1 nM	0.31 ±0.11 (n = 8)	0.97 ± 0.21 (n = 6)	p = 0.01
1 nM	0.47 ± 0.09 (n = 8)	0.90 ± 0.13 (n = 6)	p = 0.01
10 nM	0.65 ± 0.07 (n = 8)	0.83 ± 0.10 (n = 6)	p = 0.28
TRPV1 activation	Before Histamine	After Histamine	Paired T-test
	0.42 ± 0.08 (n = 7)	1.08 ± 0.26 (n = 7)	p = 0.016

n = number of subjects

Table 2 Activation of mouse DRG sensory neurons		
TRPV1 activation ($\Delta[Ca^{2+}]$ (µM))		
Krebs	+ Histamine	+ Histamine + Pyrilamine
0.03 ± 0.01 (n = 126)	0.11 ± 0.02 (n = 85)	0.02 ± 0.01 (n = 109)
(p = 0.0004)[1]		(p = 0.0016)[2]
Rectal biopsy supernatant (F340/380)		
HV supernatant	+ Histamine	Unpaired T-test
2.45 ± 0.24 (n = 32)	3.98 ± 0.24 (n = 46)	p < 0.0001
IBS-D supernatant	+ Pyrilamine	Unpaired T-test
3.90 ± 0.18 (n = 63)	2.73 ± 0.20 (n = 65)	p < 0.0001

n = number of neurons
[1] Krebs vs. + Histamine
[2] +Histamine vs. + Histamine + Pyrilamine